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ABSTRACT

Somatic karyokinesis in the hyphae of Aspergillus nidulans can be divided into at least two distinct chromosome cycles. The first, or Juvenile cycle, is a mode of karyokinesis which is restricted to young fast growing hyphae. During resting stage the nucleus in the conidium is contracted and irregular to spherical in shape. On incubation the nucleus unfolds and forms a nuclear filament to which a terminal extra-chromosomal body is attached. The nuclear filament is particulate in nature containing approximately nine Feulgen-positive bodies. It is assumed that these chromatin bodies represent the eight chromosomes of A. nidulans together with a DNA-containing centriole. The nuclear filament can attain several configurations such as linear filamentous, crescent- or horseshoe- shaped, and ring-shaped. It is believed that crescent-, horseshoe-, and ring-shaped nuclei are nuclear filaments wound around a disc-like "central body" (nucleolus), and that these configurations arise as a result of nuclear streaming and nucleolar development.

Prior to nuclear division, the centriole divides. In ring-shaped nuclei, rotation of the daughter centrioles through 180° can be observed. After the centrioles have taken up polar positions the nuclear filament divides. No spindle apparatus can be detected and it is thought that cytoplasmic streaming forces separate the daughter nuclei. After division the daughter nuclei can be either filamentous, crescent- or ring-shaped. Subsequent nuclear divisions occur in rapid

succession without intermediate resting stages. After 14 hours of incubation the nuclei condense into a resting-stage-like structure. On further incubation this structure enlarges and yields an extremely large nuclear filament which belongs to the second chromosome cycle (Maturation cycle). This cycle provides the nuclear material necessary for the development of special vegetative structures such as the sterigma and conidia.

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INTRODUCTION

The genetic analysis of linkage in the Ascomycete, Aspergillus nidulans by means of somatic crossing over has been well established (Pontecorvo, 1953; Käfer, 1958). The results of these studies, however, have not been linked to cytological observations. It has generally been taken for granted: "that mitosis in fungi follows the same course as in higher organisms" although "cytologically acceptable evidence for this belief is hard to find," (Robinow and Bakerspigel, 1965, p. 119).

The recent discovery in Neurospora crassa, another Ascomycete, of at least three different somatic chromosome cycles (karyokinetic cycles), and the observation that somatic recombination takes place in this fungus (Weijer and Dowding, 1960; Weijer et al., 1965), justify an intensive reinvestigation of somatic karyokinesis in other Ascomycetes that are known to exhibit a parasexual cycle.

Although there is no direct evidence that the vegetative karyokinetic events in Neurospora facilitate genetic exchange in a heterokaryon, the filamentous nucleus with its longitudinal mode of division as outlined by Weijer et al. (loc. cit), seems to offer a more acceptable explanation for the occurrence of the parasexual cycle than one based on classical mitosis. Since both Aspergillus and Neurospora are functionally coenocytic (Moore and McAlear, 1962), the question arises whether or not this filamentous mode of division applies to all coenocytic organisms exhibiting the parasexual cycle.

Despite the fact that the chromosomes of Aspergillus nidulans are extremely minute during the first 14 hours of conidial germination, (the Juvenile cycle), karyokinesis of the vegetative nucleus of Aspergillus nidulans can be studied with relative ease, due to the mononucleate nature of its conidium. The DNA-specific Feulgen staining technique was employed throughout the investigation, thereby, leaving no doubt as to the chemical nature of the visible structures.

LITERATURE REVIEW

From the literature it becomes evident that our knowledge of the nuclear cytology of the *Aspergilli* is rather incomplete. This is especially true of *Aspergillus nidulans*, an organism so important to genetics. The few studies dealing with nuclear division in the *Aspergilli* mainly describe chromosome cycles in the reproductive structures of these fungi. Almost no information exists on the cytology of the hyphal nuclei.

Dangeard (1907) described the conidiophore and perithecia (cleistothecia) of *Sterigmatocystis nidulans* Eidam (an early generic name for *Aspergillus nidulans*). Although he found that the primary and secondary sterigmata and the conidia were uninucleate, no description of somatic nuclear division was given. Unfortunately, Dangeard did not mention the methods of fixation and staining employed.

Frazer and Chambers (1907) studied the cytology of *Aspergillus herbariorum* Wiggens (=*Eurotium Aspergillus glaucus* DeBary). The material was fixed in dilute Flemming's solution, stained with Flemming's triple stain or Heidenhain's iron haematoxylin coupled with a solution of erythrosin in clove oil. Unsectioned material was stained in Ehrlich's haematoxylin. Both, mature conidia (containing four nuclei) and ascospores (containing 8 nuclei), were found to give rise to multinucleate mycelia. The details of divisions, however, were not described.

Dale (1909) investigated the cytology of *Aspergillus repens* de Bary fixed in Flemming's solution or chrome-acetic acid, and stained with aniline-gentian-violet followed by eosin in clove oil. The vegetative mycelium was

found to be multinucleate and the conidia were described as "containing about a dozen nuclei of different size" (p. 219). In the young ascospores, "several stainable bodies" (p. 223) were described, which condensed in the mature ascospore, to form one nucleus.

Wakayama (1931) studied vegetative nuclear division in the sterigmata of several species of Aspergilli (not including A. nidulans). In his study, material was fixed with Allen's modified solution of Bouin's fluid, sectioned, and stained with Heidenhain's iron-alum haematoxylin. According to Wakayama, nuclear division in the uninucleate sterigmata of the species studied, was "quite the same in all essential features as it is in higher organisms" (p. 299). He noticed, however, that in the resting stage the nucleus of the sterigma was characterized by a large stainable body which he identified as a karyosome. Early in mitotic nuclear division the stored chromatin became separated from the karyosome and developed into a definite number of chromosomes (n = 2). After the escape of the chromatin substance, the remaining portion of the karyosome (viz. the nucleolus) often disappeared. In rare cases, however, Wakayama found that the nucleolus remained in the spindle until the end of mitosis. Moreover, in the resting nucleus, the centrosome or centriole was part of the karyosome. Early in mitosis the centrosome escaped from the karyosome and divided into two bodies, between which an intranuclear or a central spindle developed. On completion of mitosis, each centrosome seemed to be mixed with the daughter genome at the spindle pole and a new karyosome was constituted.

Whelden (1940) described somatic nuclear division in A. niger, fixed with various solutions and stained with Heidenhain's iron-alum haematoxylin. Sterigmata as well as conidia were found to be uninucleate. The conidial nucleus divided at germination or shortly after the germination tube developed. As the proto-hypha elongated, the nuclei divided in rapid succession. Two 'chromosome pairs' were seen at anaphase in all but two germinating conidia. In the latter two conidia, a chromosome number of eight was observed. Although Whelden (loc. cit.) observed "anaphases" in his preparations, the mode of nuclear division in sterigmata, conidia, and hyphal tips, remained undiscussed.

Olive (1944) studied the development of the perithecium (cleistothecium) of Aspergillus Fischeri Wehmer, fixed with formalin-acetic alcohol and stained with iron-alum haematoxylin. Olive's description of the resting nucleus of the vegetative hypha of the cleistothecia agrees with Wakayama's (loc. cit.) observation of the resting karyosome of the sterigma. However, no further information was offered on the actual division and structure of the nucleus. Olive (1953), in a later review, attributed his earlier findings to improper fixation and staining technique, and stated that, "Apparently, as the result of a peculiar sensitivity of these nuclei to most fixatives, the chromatin collapses around the nucleolus and both stain as one body" (p. 484-485). He concluded, however, that in properly fixed preparations a nuclear reticulum could be seen which was associated with the formation of the chromosomes. However, Pinto-Lopes (1948), in his discussion of the nuclear structure of the Fungi, pointed out that, too often, the difficulty of observation on account of the smallness of the nucleus, had led "the investigators to look for several techniques which should reveal the pre-

sence or absence of nuclei. But often a technique which is good for revealing the presence of the nuclei is not equally good for the study of their structure. An important point in this connection is that in the majority of the cases, iron haematoxylin has been employed as the nuclear stain. As is well known, the intensity of the coloration to be obtained with this stain is determined by the observer himself, according to the differentiation. Of the other colorations used, among those most worthy of mention is that due to the Feulgen reaction" (p. 195-196). He further states, "The conclusion is that, while iron haematoxylin is useful in demonstrating the presence of nuclei, a more precise method, such as the Feulgen reaction, is necessary when it is attempted to study the nuclear structure in the Fungi" (p. 197).

Baker (1945) investigated the cytology of conidial formation and germinating conidia in four species of *Aspergilli* fixed in Bouin's fluid and stained with Heidenhain's iron-alum haematoxylin. In three species of *Aspergillus*, (*A. clavatus*, *A. fumigatus* and *A. repens* respectively) uninucleate phialides (viz. sterigmata) and conidia were observed. The observations reported agreed with those made earlier by Wakayama (*loc. cit*). on conidial formation in the same species. Again, no observations on the actual mechanics of nuclear division was given in this publication. Baker (*loc. cit.*) also discussed a fourth species, *A. echinulatus* (which together with *A. repens* belongs in the *A. glaucus* group). In this latter species the conidia and phialides were found to be multinucleate. In describing the formation of the conidia, Baker (*loc. cit.*) mentioned that nuclear divisions were infrequently observed in the phialide. Divisions in conidia,

however, were frequently noted. In A. fumigatus the nucleus divided once before germination, whereas in the other three species, many nuclear divisions prior to germination were observed. The young hyphae of all four species were found to be initially coenocytic and contained many small nuclei.[1] Later in their development, septa were formed which gave rise to conidiophore initials (and subsequently to conidiophores each containing many small nuclei).

Yuill (1950) studied the distribution of uni- and multi-nucleate conidia of 78 species among the 14 groups of Aspergilli. Conidia were fixed in La Cour's 2 B.D. or in Allen's Bouin solution and stained in La Cour's modification of Newton's Crystal Violet method. A uni-nucleate condition was reported for the conidia of A. nidulans.

Pontecorvo (1953) studied the cytology of A. nidulans with freshly squashed preparations stained in aceto-lactic-orcein. He provisionally concluded from observations on zygotes, that there were "four chromosome pairs--one of these is very large, one very small, and two of medium length" (p. 149). As to the nuclei in the mycelium, Pontecorvo (loc. cit.) remarked that "a direct cytological examination is of no avail because of the smallness of the chromosomes. But we may take it that from the haploid nuclei of the ascospore the nuclei of the mycelium derive by mitosis, and from these the nuclei of the conidia" (p. 148).

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[1] Although only camera lucida drawings are presented in Baker's paper, the diameter of the nucleus has been calculated by the author as being 1/10 of the hyphal diameter, or .3 μ.

Elliott (1956) described 4 bivalents at first metaphase of meiosis in a diploid strain of *A. nidulans* and thus he supported Pontecorvo's (*loc. cit.*) observation of n = 4.

Käfer (1958) established 8 definite linkage groups in *A. nidulans* by mitotic recombination analyses of 40 genetic markers.

Elliott (1960) studied the cytology of haploid and diploid strains of *A. nidulans* fixed in acetic alcohol and stained with aceto-orcein. Although no observations were made on hyphal nuclei, a detailed description of the (n = 8) chromosomes during meiosis and subsequent mitotic divisions is given. The late anaphase of all ascogenic mitotic divisions exhibited rings "in which the individual chromosomes cannot be distinguished, and a thread, presumably representing the spindle, often connect them" (p. 465). Elliott, however, stated that due to the staining method employed, he was unable to demonstrate the existence of centrioles.

MATERIALS AND METHODS

Preparation of Specimen

The wild-type haploid strain of Aspergillus nidulans, UAMH 798* was used throughout the present study.

The medium employed for maintenance of stock cultures and incubation broth was Vogel's minimal medium supplemented with trace elements and biotin, as described in "Stanford Neurospora Methods" (1963). The use of biotin in the medium was incidental.

Partial synchrony of conidial germination was induced by keeping conidiating cultures on agar slants at 4°C for at least three days prior to use. Sterile distilled water (200 ml) was used to suspend the conidia of six conidiating cultures. The suspension was filtered through a glass wool filter, to remove hyphal fragments, and diluted into minimal medium broth (1000 ml. final volume). The suspension was then divided into two 1000 ml. Florence flasks and after taking the initial 30 ml sample, both flasks were placed in a shaker at 30°C. Subsequent samples were taken from either flask at two hour intervals, for 14 hours. From 6 to 8 hours, however, hourly samples were taken.

The samples were centrifuged at 2000 r.p.m. for one minute and the supernatant was decanted. The pellet was then washed in distilled water twice, each washing being followed by resuspension and recentrifugation. The washed conidia were finally suspended in a quantity of distilled water

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*University of Alberta Mold Herbarium, University of Alberta, Edmonton, Alberta.

(5 to 15 ml.) depending on the volume of the sample. The fungal material was fixed by spreading the final suspension onto preheated cover slips with a Pasteur pipette, which were then dryed in a 60°C oven (30 to 45 minutes). The dried cover slips were then stored at room temperature.

Staining and Mounting of Preparations

The Feulgen stain was prepared as follows:

Basic fuchsin	5 g.	
$Na_2S_2O_5$	9.5 g.	
0.15 N HCl	500 ml	(room temperature)

The resulting solution was shaken for two hours. Two and a half grams of activated carbon were then added, and the suspension was shaken for two minutes after which the carbon was filtered out. The filtrate was stored at 4°C in an air tight amber bottle.

The preparations to be stained were first hydrolysed in 1N HCl at 60°C + 1°. The period of hydrolysis varied from 4 to 8 minutes for non-incubated conidia and up to 8 to 12 minutes for material incubated for 14 hours. All the preparations were stained in Feulgen for approximately 90 minutes at room temperature, after which they were rinsed in lukewarm tap water for 4 minutes or until the water cleared. The specificity of the Feulgen staining technique was checked with the use of DN-ase (Darlington and La Cour 1960). Four of the 10 hour preparations were treated and four were used as a control. The hydrolysis and staining were carried out as above.

Temporary water-mount slides were made of the initial and two hour samples. Pictures were taken of these slides within the hour. Slides from the 4, 6 and 7 hour samples were mounted directly in Gurr's Water Mounting Medium. Slides from the 8, 10, 12 and 14 hour samples were mounted directly in Gurr's Water Mounting Medium neutralized to pH 6.5 with NaOH, or were dehydrated in absolute ethyl alcohol for 15 seconds, after which the material was mounted in Euparal (1 part Euparal : 1 part Euparal Essence).

Microphotography

Observations and photomicrographs were made with a Leitz Ortholux II microscope fitted with a Leitz plano objective (100X, N.A. 1.32), Leitz periplan 12X or 10X compensating oculars or 10X periplan hi-point compensating oculars, and a Leitz Aplanatic condenser fitted with oil cap (N.A. 1.40). A Leitz Xenon lamp was used as a light source. Suitable grey filters and a 576 μ interference filter were used for the adjustment of the light intensity and for obtaining maximum contrast and resolution.

Photomicrographs were taken with either a Leitz Orthomat 35 mm automatic camera, or a Leitz Aristophot bellows camera with a Leitz 8X photo-ocular. The image distance employed with the latter was 350 mm.

Kodak High Contrast Copy Extreme Resolution Panchromatic 35 mm film was used in the Orthomat camera, and Kodak Contrast Process Ortho film (8.3 X 10.8 cm) in the Aristophot. The High Contrast Copy film was developed with Kodak D76 developer as a compensating developer. The

Kodak Ortho film was developed with Kodak D11 developer. The enlargements were preinted on Kodak, Ansco and Agfa paper of suitable gradations and developed in Kodak Dektol developer.

OBSERVATIONS

The resting conidia of the Aspergillus nidulans used in this study, are globose, rugulose, dark green in mass, 3 to 3.5 μ in diameter and uninucleate (Plate II). Binucleate conidia (4-5 μ in diameter) may be observed (Plate I), but only rarely. The resting nuclei vary greatly in size (0.5 to 1.0 μ), are usually irregular in shape, but sometimes appear to be almost spherical (Fig. 2). In the irregularly shaped nuclei, particulate bodies are sometimes noted (Figs. 4 and 9), and occasionally a DNA-positive extra-chromosomal body almost 0.2 μ in diameter can be detected (Fig. 3). The conidial wall appears slightly Feulgen-positive at all stages, and remains so, even after DN-ase pre-treatment (see p. 10).

Examination of conidia following two hours of incubation (Plate III) shows that some of the conidia have swollen. The nuclei have lost the characteristic condensed structure of the resting stage and have started to unfold (Figs. 14 and 15). Particulate Feulgen-positive bodies can be clearly seen in many of the conidia (Figs. 10, 11, and 16). Nuclear filaments (Figs. 12 and 13) varying in length from 1.5 to 7μ, are often encountered. An extremely dense Feulgen-positive particle can sometimes be seen at one end of the filament, and in a few conidia this body is bi-partite (Fig. 13). Nuclear ring configurations (Figs. 10 and 17) and horseshoe-shaped configurations (Fig. 16) occur with very low frequency at this stage of development. A dense Feulgen-positive body connected to these configurations, can usually be observed.

The nucleus, after four hours of incubation, is still contained within the conidium (Plate IV). The conidia are now 4-5 μ in diameter and

breaks in the conidial wall can be seen. (Figs. 18, 19 and 21). Infrequently, germinated conidia are observed and these usually contain a divided nucleus (Fig. 24). Most of the nuclei encountered are filamentous, 4 to 5 μ in length, and consist of particulate Feulgen-positive bodies (Figs. 18 and 20). Some of these nuclear filaments have a 'horseshoe-like' structure (Figs. 19, 21, 22 and 25), and others have a ring-shaped structure (Figs. 23 and 24). A densely Feulgen-positive particle is sometimes seen at one end of the filament (Fig. 20) or connected to the ring (Fig. 24).

The six hour cultures (Plate V) contain relatively large numbers of nuclear ring configurations 1.0 to 1.5 μ in diameter (Figs. 29, 32 and 35). These remain particulate in nature with dense Feulgen-positive body averaging 0.3 to 0.5 μ in diameter contained either within the ring (Fig. 35) or attached to it (Fig. 29). Division of this structure together with the rotation of its division products around the nucleus, can be observed in detail (Figs. 29, 32 and 35). Open rings are sometimes seen (Figs. 28 and 34). Due to their less contracted nature, these were used for counting the number of Feulgen-positive particles within the conidium. A maximum of nine particles can be counted in most of these structures (Figs. 33 and 34). A unstained region can sometimes be detected within the ring-like nucleus (Fig. 28).

After seven hours of incubation, (Plate VI) germination tubes 3-15 μ long are encountered. Although many ring and filamentous nuclei are still undivided at this time (Figs. 36, 38 and 40), post-divison configurations have increased in frequency in comparison with the six hour

preparations (Figs. 37, 41, 42, 43 and 44). A dividing filamentous nucleus in the mycelium often gives rise to a Y-shaped configuration (Fig. 39). Division of a ring or a horseshoe-shaped nucleus may also give rise to two similar daughter nuclei (Figs. 42, 43, and 44). Other variously shaped post-division nuclei are often noted. (Fig. 37). The extra-chromosomal bodies, which divided prior to nuclear division, are seen after karyokinesis as dense Feulgen-positive bodies either located at the terminal end of the daughter filament (Fig. 39), or associated with the daughter ring (Fig. 43). The ring is sometimes found to have two overlapping free ends (Fig. 42), indicating that the structure is not continuous. Hyphal growth is observed to slow down during nuclear division (Figs. 33, 38 and 41; also Plate IV, Fig. 24).

Nuclear configurations similar to those described in the preparations from six and seven hour cultures are encountered with greater frequency in the eight hour cultures (Plates VII, VIII, IX and X). Predivision ring nuclei (Figs. 58 and 59) and daughter ring nuclei (Figs. 54 and 56), as well as filamentous nuclei in different stages of division, (Figs. 47, 48, 49 and 64) are often detected. Double filaments, (Fig. 47) and filaments in the process of separation (starting from the extra-chromosomal end) are also frequently noted (Figs. 48, 50, 64 and 49). Filaments are often observed to be in horseshoe or crescent shapes (Figs. 47, 51, 53, 55, 57, 61, 64 and 65). Odd shaped nuclear configurations such as: twisted and dividing filaments (Fig. 62), "S-like" structures (Fig. 63), and "Figure-eights" (Figs. 52 and 66) are often seen. Usually one, and sometimes both, of the daughter nuclei move out of the conidium after division (Figs. 53, 54 and 56).

After 10 hours of incubation a great many nuclei are found in the young hypha (approx. 1 nucleus per 6 μ of hyphal length; Fig. 68), indicating that repeated division has taken place between eight and ten hours of incubation. The nuclei are usually in a ring configuration 1.2 to 1.8 μ in diameter (Figs. 68, 69 and 70), although filaments are also present (Fig. 68). Ring structures are sometimes found to give rise to filaments and rings (Figs. 68). Synchrony in nuclear division within a hypha is readily detected at this stage (Fig. 68). The extra-chromosomal body is either incorporated into the nuclear ring (Fig. 70) or attached to it (Fig. 68).

After twelve hours of incubation the hyphae have become extremely long and there is further evidence that repeated nuclear division has taken place (Plate XII). Nuclear rings (Figs. 71, 74 and 75) as well as dividing filamentous nuclei (Figs. 72 and 73), approx. 4.5 μ in length, are frequently observed. Dividing ring nuclei are sometimes seen to give rise to a daughter ring and a daughter filament (Figs. 71, 73 and 75).

Preparations from 14 hour material (Plate XIII) show nuclei in various stages of condensation (Figs. 76-81 incl.). In some hyphae the nuclei are extremely condensed (4.5 to 6.8 μ in diameter) and appear to be almost spherical (Figs. 79 and 80). The extra-chromosomal body is often found to accompany these nuclei, but it is somewhat less dense and is farther from the main nuclear mass than previously observed (Fig. 80). Very infrequently, filaments are seen which are very much larger (in terms of total nuclear volume) than those noted in earlier preparations (Fig. 82). These filaments exceed 10.5 μ in length, and consist of nine Feulgen-positive bodies, one of which is terminally located and triangular in shape.

DISCUSSION

Robinow and Bakerspigel (1965) in their recent review on somatic nuclei and forms of mitosis in fungi wrote: "Today it appears to us that fungal cytology faces three principal problems: (1) the basis of the commonly encountered low affinity of somatic fungal chromosomes for ordinary stains if these are used in ordinary ways; (2) the meaning of the peculiar associations which the chromosomes of some species form at metaphase; (3) the nature of the forces or devices which separate sister chromosomes at anaphase. In other words, the frequent absence of a demonstrable spindle apparatus" (p. 120).

Although these authors in their particular fungal studies were faced with these problems, their generalizations as applied to the entire field of fungal cytology, does not seem to be justified (Raper and Esser, 1964). However, even though our knowledge of nuclear phenomena in the fungi is still very limited, conclusive evidence has been produced that the mode of somatic karyokinesis differs in some of the coenocytic Ascomycetes from the one encountered in fungi with a typical cellular organization (Robinow and Bakerspigel, 1965). In _Neurospora_ _crassa_ (Weijer _et_ _al_., 1965), three different subsequent modes of karyokinesis occur during vegetative growth, all characterized by the absence of a "classical" spindle apparatus. It appears, therefore, that the real problem in fungal cytology is whether these different chromosome cycles have any direct relationship with classical mitosis, or whether they constitute an entirely different mode of karyokinesis related only in the ancestral sense to the nuclear phenomena of higher plants (Weijer _et_ _al_., 1965).

As will be discussed later (p. 21), the somatic karyokinesis (Juvenile cycle) of the coenocytic Ascomycete, *A. nidulans* does not differ in essence from the one studied in *Neurospora crassa* (Dowding and Weijer, 1960, 1962; McDonald and Weijer, 1966; Weijer, 1964; Weijer and Dowding, 1960; Weijer and Koopmans, 1964; Weijer et al., 1963, 1965; Weijer and McDonald, 1966) and in *Gelasinospora tetrasperma* (Dowding and Weijer, 1962 and Weijer *et al.*, 1963). In both organisms the mode of division is "non-classical".

As Pinto-Lopes (1949) pointed out in his discussion of the nuclear structure of the fungi, the extreme smallness of the nuclei often demands the use of nuclear stains which may be useful for identification of the nucleus, but which may be completely unsuitable for critical studies of its structure. In those cases in which a differential staining technique (i.e. iron haematoxylin, crystal-violet, propiono-carmine, etc.) has been empolyed, the interpretation of nuclear structure is greatly influenced by the degree of differentiation of staining intensity. "While iron-haematoxylin is useful in demonstrating the presence of nuclei, a more precise method, such as the Feulgen reaction, is necessary when it is attempted to study the nuclear structure in the Fungi" (Pinto-Lopes, *loc. cit.*, p. 197).

From the existing literature it becomes evident that the identification of stainable bodies in *Aspergillus conidia* (according to structure) has not always been very successful. Neither Dangeard (1907), Frazer and Chambers (1907), Dale (1909) nor Baker (1945) claimed to be able to distinguish adequately between a nucleus and a chromosome. Dale (*loc. cit.*) however, mentioned that in mature conidia "several stainable bodies" condense into a larger aggregate, and it might very well be that an explanation based

on chromosome-like structures was intended. Additional support for such a view might be found in the recorded observations in three species of the Aspergilli (A. clavatus, A. repens, and A. echinulatus) in which many nuclear divisions take place before conidial germination (Baker loc. cit.). More recent studies (Tanaka et al., 1965) seem to contradict the possibility of the occurrence of more than one nuclear division prior to germination of the conidium. The latter authors were unable to prove an increase in DNA synthesis during the initial stages of germination. This would indicate that the pre-requisite for Baker's claim of several nuclear divisions prior to germination of the conidium had not been met. The present study interprets the appearance of a "multinucleate" conidium (in A. nidulans) as being due to the unfolding of the single nucleus into a composite structure in which the components are physically attached to one another.

An attempt to count chromosomes was carried out by Wakayama (1931), Whelden (1940) and Olive (1944). In all these studies 'two pairs of chromosomes' were identified at anaphase of the sterigmal nucleus. In addition, Whelden (loc. cit.) investigated the chromosome number of 'anaphase' nuclei of germinating conidia. Again, 'two chromosome pairs' were reported, however, in a few cases he observed four pairs of chromosomes (i.e., eight chromosomes). The present study supports the view that eight chromosomes make up the complete haploid nuclear compliment, (Käfer, 1958; Elliot, 1961) although in the majority of nuclei the number of chromatin bodies observed was less than eight.

The cytology of the somatic nucleus (not including the somatic nuclei of the ascus) can be sharply divided into a Juvenile cycle, which

gives rise to the multinucleate and undifferentiated hypha, and one or more Maturation cycles which are involved in the development of specific vegetative structures, such as sterigmata and conida. The Juvenile cycle is operative for approximately 14 hours under the conditions employed. During this time an extensive hyphal structure develops. After 14 hours, nuclear behavior changes course. No longer are ring divisions encountered, and the nucleus condenses into a spherical body, which upon further incubation gives rise to a long filamentous nucleus (Figs. 76-82). The transition from Juvenile to Maturation cycle occurs at this point of hyphal development (Weijer *et al*., 1965). The large nuclei pictured in Fig. 80 are identical to those studied by Weijer *et al*., (*loc. cit*.) in *Neurospora crassa*. Although not visible when stained with Feulgen, a large nucleolus is present (Weijer *et al*., 1965; crystal violet staining) and the nucleus, nucleolus and centriole, appear to be contained in one and the same structure. Wakayama (1931) and Olive (1944) found (in *Aspergillus*) that this structure (Global-phase or karyosome) is the beginning of a chromosome cycle which is involved in the formation of the sterigmata.

Olive's (1953) statement, that the peculiar structure of the karyosome in fungi is caused by the collapse of the chromatin structure and is therefore merely a fixation artifact, appears to be unfounded. The recent *in vivo* observations of Weijer *et al*., (1963) of Global phase in *N. crassa* have unequivocally confirmed Wakayama's observation of a karyosome.

Although there are many differences in the mode of karyokinesis between the Juvenile cycle and the Maturation cycle (Weijer *et al*., 1965), the obvious and most important difference between these two, concerns the

increased polytenic nature of the Maturation nuclear configuration. The total chromosomal volume of such a configuration exceeds many times that of the nucleus of the nucleus of the Juvenile chromosome cycle.

In essence, there are no major differences between the Juvenile chromosome cycle of *A. nidulans* and the Juvenile chromosome cycle of *N. crassa*. In *A. nidulans* this cycle is characterized by the smallness of its chromosomes, an observation which led Pontecorvo (1953) to remark that "a direct cytological observation is of no avail" (p. 148). The present author concurs with the observation but not necessarily with the conclusion.

Germinating conidia stained with Feulgen reveals eight or nine extremely small Feulgen-positive bodies. Three of the chromatin bodies have a diameter corresponding to the limit of optical resolution (0.2 μ), while the other five are somewhat larger in size (0.3 - 0.35 μ). A densely stained Feulgen positive body was often observed to be associated with the nucleus at different stages of development. Hence, occasionally nine Feulgen-positive bodies were resolved. In the large pre-division filament of the Maturation cycle (Fig. 82) this ninth body is terminally located and triangular in shape. Weijer *et al.* (1965) found in *N. crassa* a similar Feulgen-positive triangular body, which was associated with the hyphal nucleus. The latter authors concluded that this extra-chromosomal body constituted the centriole and not a chromosome. Later studies by McDonald and Weijer (1966) confirmed the presence of a DNA containing centriole during nuclear division.

The fact that the centriole is Feulgen positive (and hence contains DNA) is not surprising in view of the observations that other self-duplicating extra-nuclear bodies such as mitochondria and chloroplasts also contain DNA (Schatz et al., 1964; Chun et al., 1963). On the basis of their similarities in staining and behavior during mitosis, Schrader (1936) and Darlington (1937) believe that the centromere and the centriole are homologous. Pollister (1939) and Pollister and Pollister (1943) provided evidence for this homology with their observations that the supernumerary centrioles in Vivipara malleatus were de facto detached centromeres. As stated above (p. 20) some variability existed in the number of chromatin bodies counted. This variability is attributed to the extremely small size of some of the chromosomes. Measurements of the smallest chromosomes of the pre-division filamentous Maturation nucleus (Fig. 82) reveals a diameter of 0.2 - 0.3 μ (the limit of resolution is approximately 0.2 μ). Hence, one can expect that the minute chromosomes of the much smaller Juvenile filamentous or ring configurations can only be detected rarely, and only in the very best of preparations.

Similarly, the solid (non-particulate) nuclear rings as depicted in Figs. 68-70, (lacking the characteristic particulate nature of the nuclear rings developed during the early stages of germination) can be explained either on the basis of insufficient optical resolution or to other causes, such as nuclear streaming and DNA replication.

Another factor which hindered observation of the complete complement of chromosomes and the centriole, was the slight Feulgen-positive condition of the conidial wall. The staining ability of the conidial wall

is most likely due to nucleic acids being incorporated into the structure of the wall (Horikoshi and Iida, 1964).

It is evident from the observations presented, that somatic karyokinesis in the Juvenile chromosome cycle of *A. nidulans* follows a pattern similar to that proposed for *N. crassa* (Weijer, D.L., 1964; Weijer and Koopmans, 1964; Weijer *et al.*, 1963; Weijer *et al.*, 1965). Since the hyphae of *A. nidulans* (excluding the sterigmata) are coenocytic in organization, it is not surprising that the nuclei belonging to one and the same lumen divide synchronously. The environmental conditions triggering nuclear division extend in this particular case over an entire cell lumen, probably over considerable hyphal distances, and may be assumed to be dependant on nuclear streaming. Hyphal growth occurs in cycles and was observed to slow down visibly during the initial nuclear division, indicating an interdependence of hyphal growth and nuclear division.

During the initial stages of the Juvenile karyokinetic cycle, the resting nucleus unfolds into either a ring, crescent (horse-shoe), or a filamentous configuration. In all these structures the chromosomes are linearly arranged in a filament. This multiplicity of different configurations during the early stage of nuclear development is probably caused by the mechanics of the nuclear ring formation. As Weijer *et al.* (1963) pointed out in their *in vivo* studies of nuclear behavior in *N. crassa*, the chromosomes seem to be arranged around a "flat disc-like central body" (p. 848). This body is Feulgen-negative and was interpreted to be the Juvenile nucleolus. Although RNA specific stains were not employed in the present study, the observations of a Feulgen-negative center in

the ring-like nucleus support this interpretation. Crescent or horseshoe-like configurations are believed to be merely intermediate stages in ring formation. At later stages of growth (12 hours), ring structures are observed much more frequently than filaments. The high incidence of ring configurations, in more mature structures, is possibly due to increased cytoplasmic streaming which may wind the filamentous nucleus around its nucleolus. It would thus be possible, as a result of decreased streaming, that a ring shaped nucleus can unwind into a filamentous one.

Studies on DNA synthesis in germinating conidia of *N. crassa* (Weijer and Koopmans, 1964), and on the radiosensitivity of karyokinetic stages (Weijer, 1964), indicated that DNA replication occurred during the late filament and early ring stages of the Juvenile cycle. Tanaka *et al.*, (1965) found in A. niger that although s-RNA and m-RNA are synthesized in the initial stages of germination, DNA is not synthesized. In the present study nuclear division was observed to occur after initial germination. Therefore, DNA synthesis must have taken place just prior to nuclear division, either during the filament or the ring stage. The apparently equal volume of DNA in the pre- and post-divisional nuclei discounts the possibility that the nucleus of the resting conidia contains DNA in a replicated state.

Although division of the centriole precedes nuclear division (Figs. 29, 32 and 35), replication of centriole DNA probably coincides with nuclear DNA replication. Direct evidence for such a view was furnished by Bucher and Mazia (1960) in their studies with eggs of the sea urchin, *Strongylocentrotus purpuratus*. They found that formation of chromosomal DNA did not depend on the prior duplication of the "mitotic centers".

In the filamentous nucleus, the centriole is located terminally, and after its division, nuclear karyokinesis is initiated. Although it is possible that a replusion force between daughter centrioles causes the separation of the daughter filaments, on the basis of *in vivo* observations in *N. crassa* (Weijer *et al.*, 1963), it is believed that cytoplasmic streaming is the main mechanism of daughter filament separation.

In ring-shaped nuclei the centrioles rotate around the ring in order to take up polar positions. This is followed by nuclear division resulting in two daughter rings. Whether or not the daughter nuclear rings (to which the centrioles are individually connected) participate in such a rotation is not known. From studies in *N. crassa* (Weijer *et al.*, 1965) it would seem that at the time of centriole rotation the chromosomes are not divided, although DNA replication has taken place. This also appears to be the case in the nuclear filament of *A. nidulans*. It seems, therefore, that the centriole behaves autonomously in its rotation around the nuclear ring.

It is assumed that before separation of the daughter nuclei can proceed, the nucleolus has to undergo division, and it is therefore possible that the actual separation of the daughter nuclei is guided by the division of the nucleolus rather than by the centrioles.

The division products of a ring nucleus can be either filamentous or ring-shaped, depending on whether or not the nuclear strand unwinds from around the nucleolus.

Nuclear division is repeated at least every forty minutes after the initial nuclear division has taken place in the germinating conidia. No nuclear resting stage has been found between subsequent divisions, and it seems that the nuclear filament as well as the nuclear ring undergo continuous division. Under the environmental conditions employed, these divisions continue for approximately fourteen hours. At this time, hyphal growth terminates due to exhaustion of the medium, and the nucleus condenses into a spherical configuration similar to that of the resting nucleus in the conidia. With this stage, the nucleus enters the initial phase of the subsequent chromosome cycle (Maturation cycle), involving the development of the sterigmata (Wakayama, 1931). The different stages of the Juvenile chromosome cycle are depicted schematically in Fig. 60.

REFERENCES

Baker, G.E. 1945. Conidium formation in species of *Aspergilli*. Mycologia 37:582-600.

Bucher, N.L.R., and D. Mazia. 1960. Deoxyribonucleic acid synthesis in relation to duplication of centers in dividing eggs of the sea urchins, Strongylocentrotus purpuratus. J. Biophys. Biochem. Cytol. 7:651-655.

Chun, E.H.L., M.H. Vaughan, and A. Rich. 1963. The isolation and characterization of DNA associated with chloroplast preparations. J. Molec. Biol. 7:130-141.

Dale, E. 1909. On the morphology and cytology of *Aspergillus repens* de Bary. Annls. Mycol. 7:215-225.

Dangeard, P.A. 1907. L'origine du périthèce chez les Ascomycetes. Botaniste 10:1-385.

Darlington, C.D. 1937. The external mechanics of the chromosomes. Proc. R. Soc. B 121:264-319.

Darlington, C.D., and L.F. LaCour. 1960. The handling of chromosomes. George Allen and Unwin Ltd., London. 248 p.

Dowding, E.S., and J. Weijer. 1960. Mitosis in *Neurospora*. Nature 188:338-339.

Dowding, E.S., and J. Weijer. 1962. Mitosis in *Neurospora* and *Gelasinospora* I. Genetica 32:339-351.

Elliott, C.G. 1956. Chromosomes in micro-organisms. Symp. Soc. Gen. Microbiol. 6:279-295.

Elliott, C.G. 1960. The cytology of *Aspergillus nidulans*. Genet. Res. 1:462-476.

Frazer, H.C.I., and H.S. Chambers. 1907. The morphology of *Aspergillus herbariorum*. Annls. Mycol. 5:419-431.

Horikoshi, K., and S. Iida. 1964. Studies on the spore coats of fungi. I. Isolation and composition of the spore coats of *Aspergillus oryzae*. Biochim. Biophys. Acta 83:197-203.

Käfer, E. 1958. An 8-chromosome map of *Aspergillus nidulans*. Adv. Genet. 9:105-145.

McDonald, B., and J. Weijer. 1966. The DNA content of centrioles of Neurospora crassa during divisions I and IV of ascosporogenesis. Can. J. Genet. Cytol. In press.

Moore, R.T., and J. H. McAlear. 1962. Fine structure of Mycota. 7. Observations on septa of Ascomycetes and Basidiomycetes. Am. J. Bot. 49:86-94.

Olive, L.S. 1944. Development of the perithecium in Aspergillus Fischeri Wehmer, with a description of crozier formation. Mycologia 36:266-275.

Olive, L.S. 1953. The structure and behavior of fungus nuclei. Bot. Rev. 19:439-586.

Pinto-Lopes, J. 1949. Contribution to the study of the nuclear structure in fungi. Port. Acta Biol., Ser. A, 2:191-210.

Pollister, A.W. 1939. Centrioles and chromosomes in the atypical spermatogenesis of Vivipara. Proc. Natn. Acad. Sci. U.S.A. 25:189-195.

Pollister, A.W., and P.F. Pollister. 1943. The relation between centriole and centromere in atypical spermatogenesis in viviparid snails. Ann. N.Y. Acad. Sci. 45:1-48.

Pontecorvo, G. 1953. The genetics of Aspergillus nidulans. Adv. Genet. 5:141-238.

Raper, J.R., and K. Esser. 1964. The fungi, p. 139-244. In J. Brachet and A.E. Mirsky (ed.), The Cell, Vol. VI, Academic Press, New York.

Robinow, C.F., and A. Bakerspigel. 1965. Somatic nuclei and forms of mitosis in fungi, p. 119-142. In G.C. Ainsworth and A.S. Sussman, (ed.), The Fungi, Vol. I, Academic Press, New York.

Schatz, G., E. Haslbrunner, and H. Tuppy. 1964. Deoxyribonucleic acid associated with yeast mitochondria. Biochem. Biophys. Res. Commun. 15:127-132.

Schrader, F. 1936. The kinetochore on spindle fiber locus in Amphiuma tridactylum. Biol. Bull. Mar. Biol. Lab., Woods Hole, 70:484-498.

Stanford Neurospora methods. In Neurospora Newsletter 4:21-25. (1963).

Tanaka, K., F. Kogane, and T. Yanagita. 1965. Is deoxyribonucleic acid of Aspergillus oryzae conidia modified chemically in the early period of germination? J. Gen. Appl. Microbiol. Tokyo 11:85-90.

Wakayama, K. 1931. Contributions to the cytology of fungi. III. Chromosome number in Aspergillus. Cytologia 2:291-301.

Weijer, D.L. 1964. Karyokinesis of somatic nuclei of Neurospora crassa. I. The correlation between radiosensitivity and their conidial karyokinetic stage. Can. J. Genet. Cytol. 6:382-392.

Weijer, J., and E.S. Dowding. 1960. Nuclear exchange in a heterokaryon of Neurospora crassa. Can. J. Genet. Cytol. 2:336-343.

Weijer, J., and A. Koopmans. 1964. Karyokinesis of somatic nuclei of Neurospora crassa. II. DNA replication in synchronously dividing conidial nuclei. Can. J. Genet. Cytol. 6:426-430.

Weijer, J., and B.R. McDonald. 1965. Karyokinesis of somatic nuclei of Neurospora crassa. IV. The occurrence of the juvenile ring phase during division IV of ascosporagenesis. Can. J. Genet. Cytol. 7:519-522.

Weijer, J., A. Koopmans, and D. Weijer. 1963. Karyokinesis in vivo of the migrating somatic nucleus of Neurospora and Gelasinospora species. Trans. N.Y. Acad. Sci. 25:846-854.

Weijer, J., A. Koopmans, and D.L. Weijer. 1965. Karyokinesis of somatic nuclei of Neurospora crassa. III. The juvenile and maturation cycles (Feulgen and crystal violet staining). Can. J. Genet. Cytol. 7:140-163.

Whelden, R.M. 1940. "Mutations" in Aspergillus niger bombarded by low voltage cathode rays. Mycologia 32:630-643.

Yuill, E. 1950. The numbers of nuclei in conidia of Aspergilli. Trans. Br. Mycol. Soc. 33:324-331.

Plate I, Fig. 1. Conidium of _A._ _nidulans_ (0 hours of incubation).

Fig. 1 -- Binulceate conidium.

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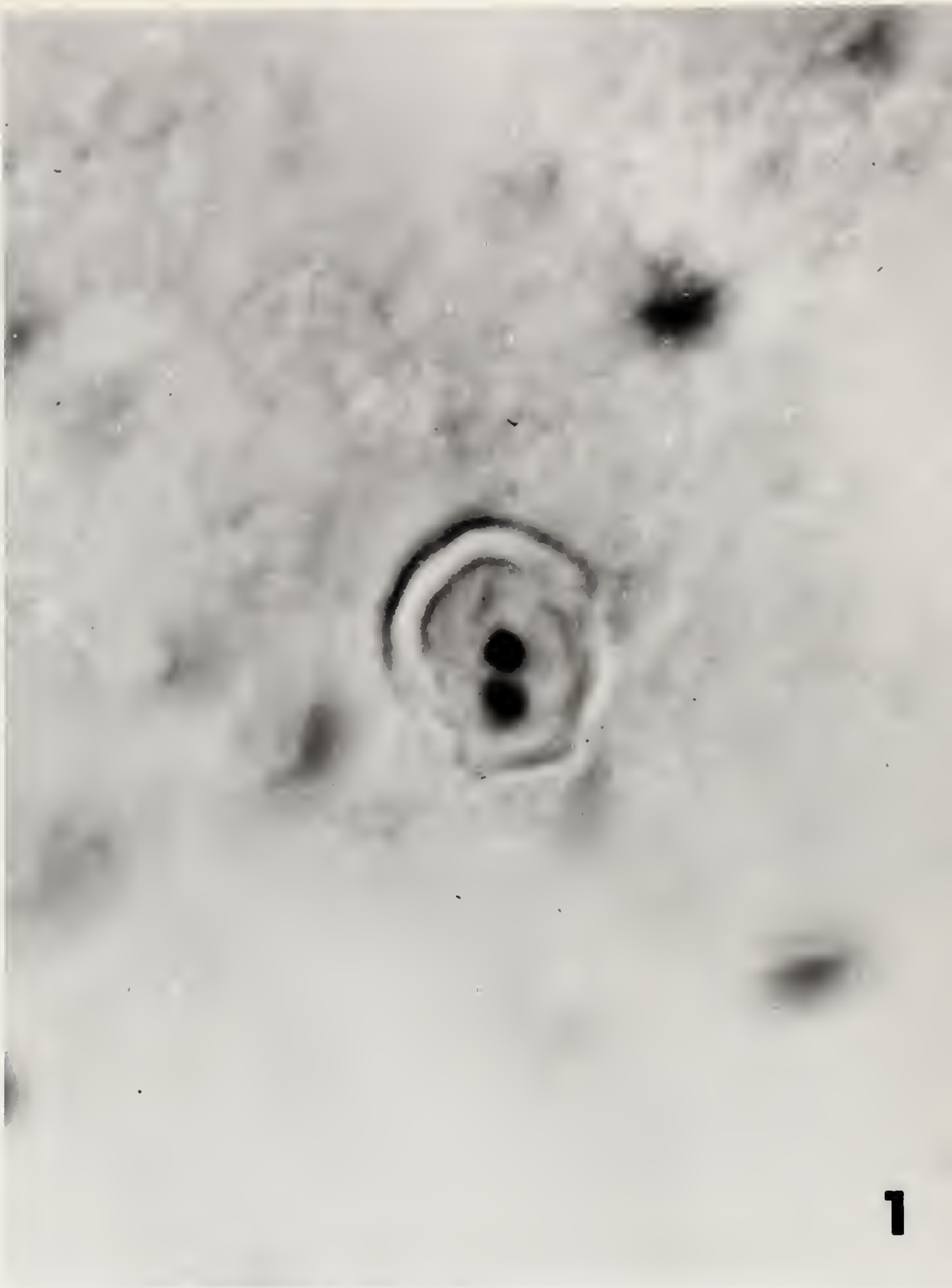

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Plate II, Figs. 2-9. Conidia of A. nidulans (0 hours of incubation)

Fig. 2 -- Conidium with a spherical resting nucleus.

Fig. 3 -- Conidium with a resting nucleus and attached centriole.

Fig. 4 -- Conidium with an irregular shaped resting nucleus.

Fig. 5 -- Conidia with irregular shaped resting nuclei.

Fig. 6 -- Conidia with resting nuclei.

Fig. 7 -- Conidia with an imperfectly spherical resting nucleus.

Fig. 8 -- Chain of resting conidia, possibly from one conidiophore chain, nuclei irregular.

Fig. 9 -- Conidia with resting nuclei. Conidium (third from left) containing four bodies.

Mag. x 4000

C = centriole

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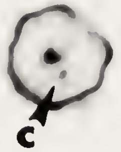

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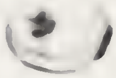

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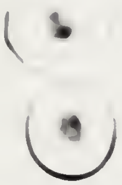

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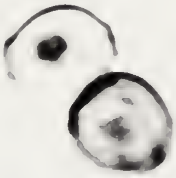

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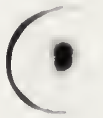

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Plate III, Figs. 10-17. Conidia of *A. nidulans* (2 hours of incubation).

Fig. 10 -- Conidia with partially unfolded nuclei. Conidium (center) with ring nucleus containing 4 chromosomes.

Fig. 11 -- Swollen conidia containing partially unfolded irregularly shaped nuclei of particulate bodies.

Fig. 12 -- Conidia with partially unfolded nuclear filaments.

Fig. 13 -- Conidium (lower) with a filamentous nucleus and divided centriole.

Fig. 14 -- Conidium with resting nucleus just starting to unfold.

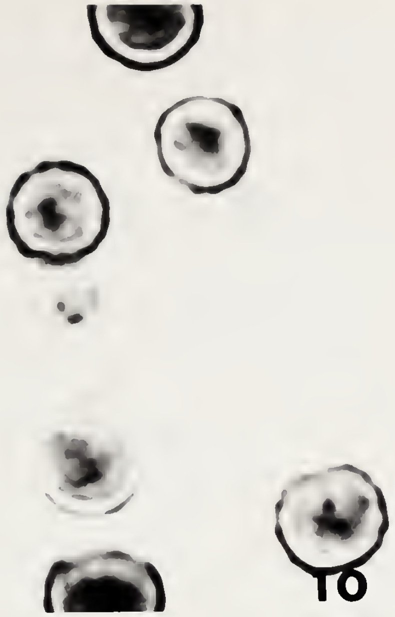
Fig. 15 -- Conidium with resting nucleus just starting to unfold.

Fig. 16 -- Conidium with early 'horseshoe-like' nucleus containing 5 stainable bodies.

Fig. 17 -- Conidium with ring-shaped nucleus of a partially particulate nature and centriole.

Mag. x 4000

C = centriole



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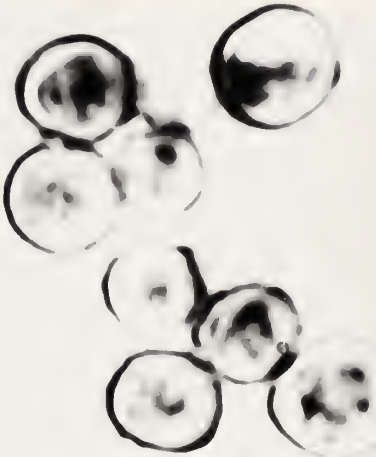

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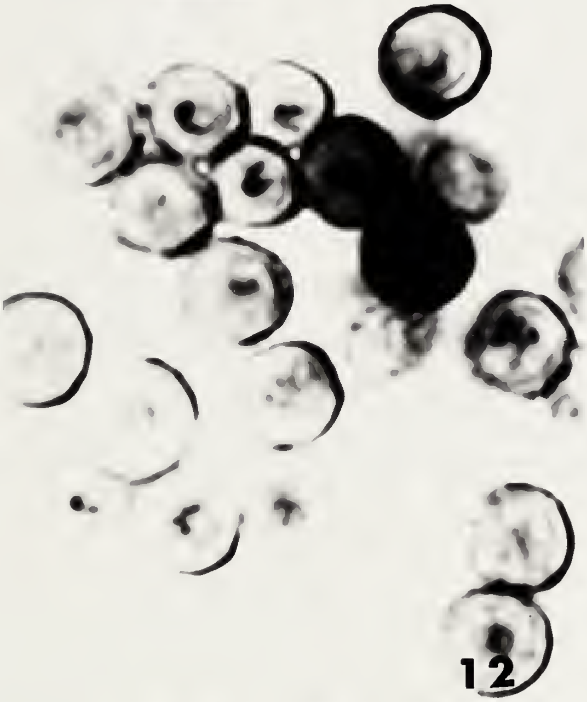

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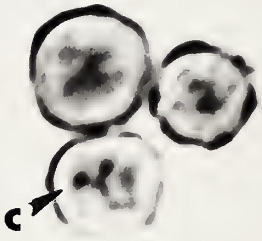

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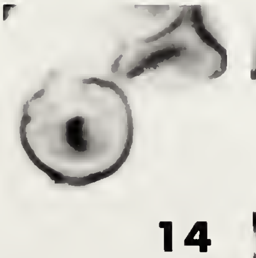

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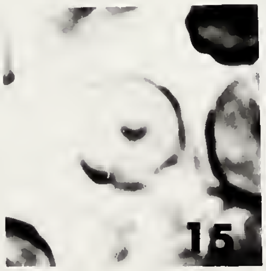

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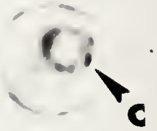

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Plate IV, Figs. 18-25. Conidia of A. nidulans (4 hours of incubation).

Fig. 18 -- Swollen conidium with break in conidial wall and with folded filamentous-like nucleus (top left). Germinated conidium with 'horseshoe-like' filamentous nucleus (center).

Fig. 19 -- Conidium (center) with 'horseshoe-like' filament of approximately 8 chromosomes and centriole.

Fig. 20 -- Conidium with 'horseshoe-like' filament and centriole.

Fig. 21 -- Conidium (center) with 'horseshoe-like' nucleus and break in conidial wall.

Fig. 22 -- Conidium (center) with crescent-shaped nucleus (containing approximately 5 particulate bodies).

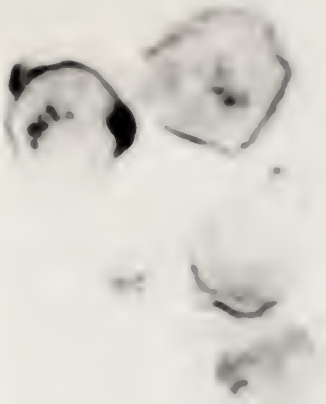
Fig. 23 -- Conidium (center) with nuclear ring of 6 chromatin bodies.

Fig. 24 -- Germinated conidia containing daughter ring plus centriole and daughter crescent nucleus. Possible binucleate conidium (bottom).

Fig. 25 -- Conidium with imperfectly shaped 'horseshoe-like' nucleus.

Mag. x 4000

C = centriole



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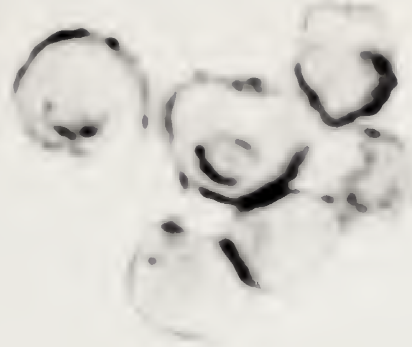

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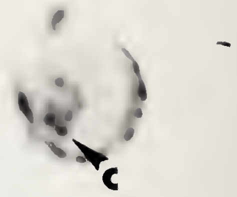

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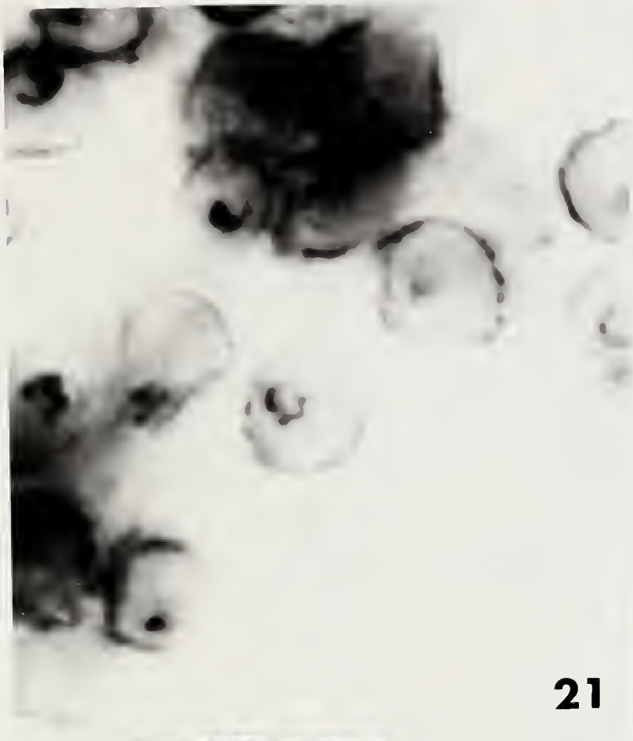

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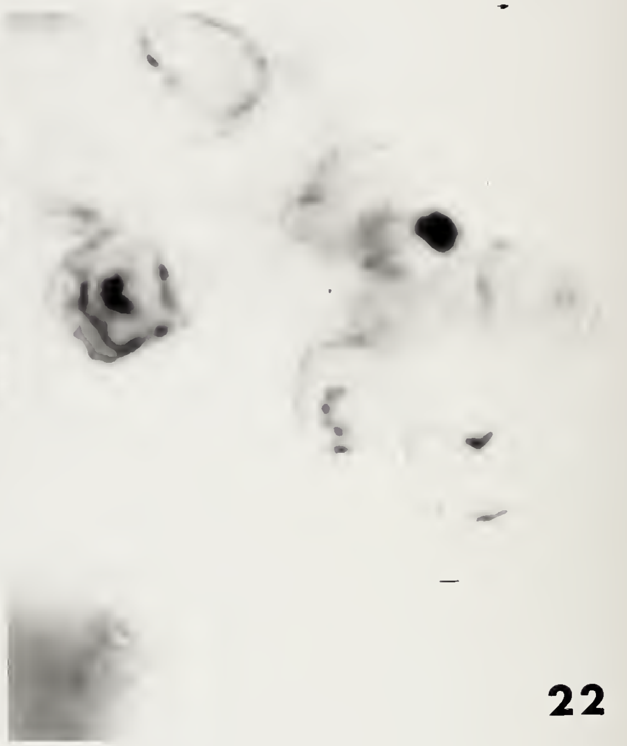

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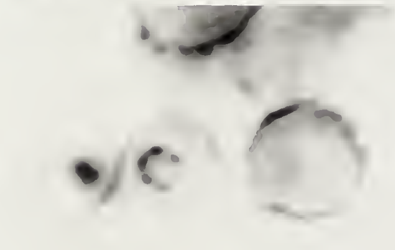

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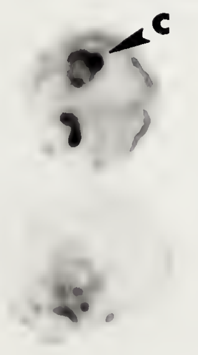

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Plate V, Figs. 26-35. Conidia of A. *nidulans* (6 hours of incubation).

Fig. 26 -- Conidium with duplicated filament.

Fig. 27 -- Conidium containing duplicated filament with triangular shaped centriole.

Fig. 28 -- Conidium with 'horseshoe-like' nucleus with microscopically empty region in the center.

Fig. 29 -- Conidium (center) with nuclear ring and attached centriole.

Fig. 30 -- Conidium with nuclear filament, possibly duplicated.

Fig. 31 -- Conidium containing 'S-shaped' filamentous nucleus.

Fig. 32 -- Conidium (left) with nuclear ring and divided centriole.

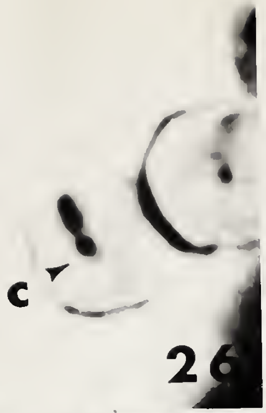
Fig. 33 -- Germinated conidium with undivided 'horseshoe-like' nucleus containing 9 chromatin bodies.

Fig. 34 -- Conidium containing 'horseshoe-like' nucleus of 9 chromatin bodies.

Fig. 35 -- Conidium containing nuclear ring structure with attached divided centrioles.

Mag. x 4800

C = centriole



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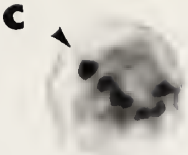

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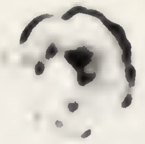

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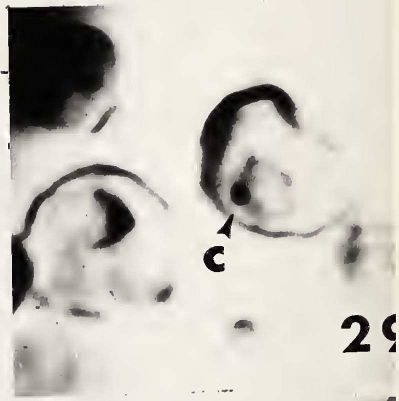

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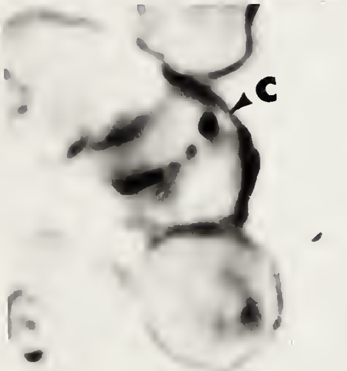

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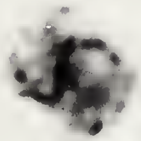

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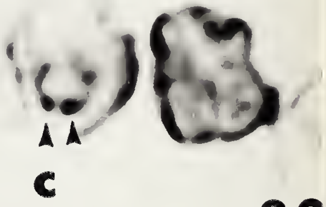

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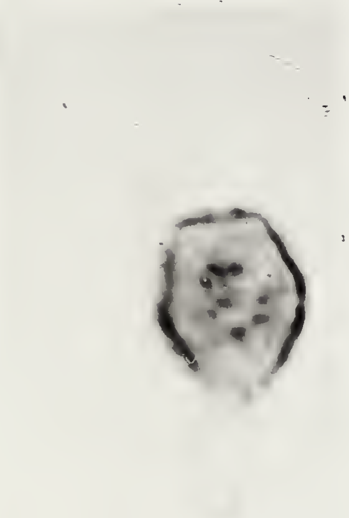

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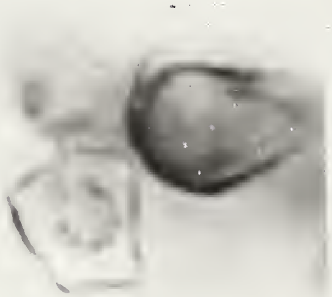

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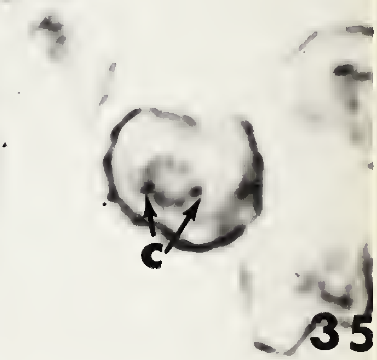

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Plate VI, Figs. 36-44. Conidia of A. nidulans (7 hours of incubation).

Fig. 36 -- Germinated conidium with undivided crescent nucleus.

Fig. 37 -- Daughter nuclei in germinated conidium.

Fig. 38 -- Germinated conidium with filamentous nucleus just starting to divide.

Fig. 39 -- Dividing hyphal filamentous nucleus in 'Y-shaped' configuration.

Fig. 40 -- Germinating conidium with ring nucleus of 8 chromosomes and centriole.

Fig. 41 -- Post-division nuclei in germinated conidia.

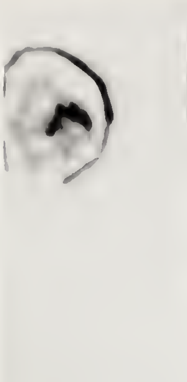
Fig. 42 -- Germinating conidium containing ring nucleus with overlapping free ends (bottom). Daughter ring nuclei (top left).

Fig. 43 -- Germinating conidium (center) containing identical daughter ring nuclei.

Fig. 44 -- Germinating conidium containing identical 'horseshoe-like' daughter nuclei.

Mag. x 4000

C = centriole



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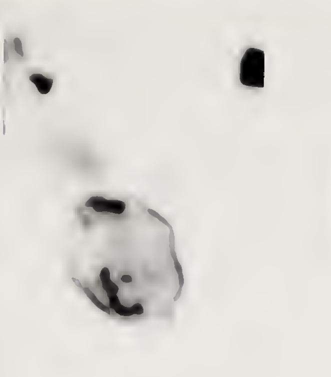

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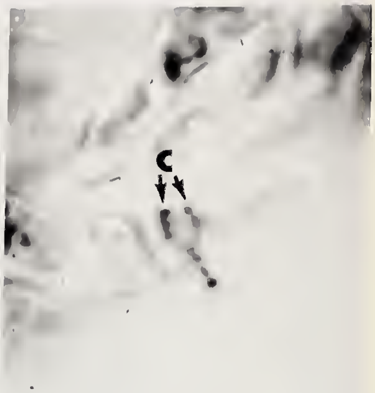

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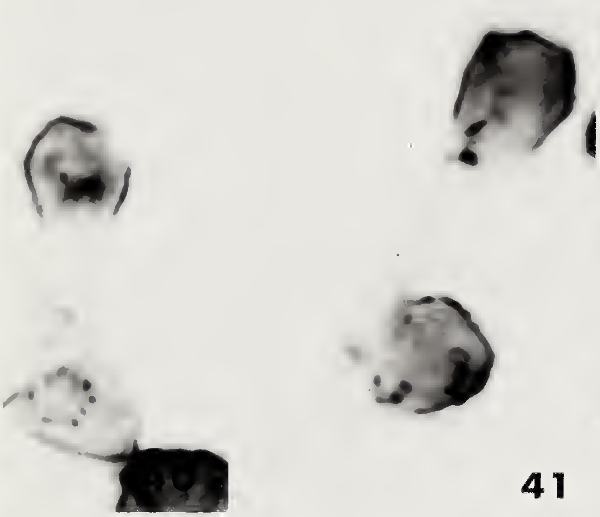

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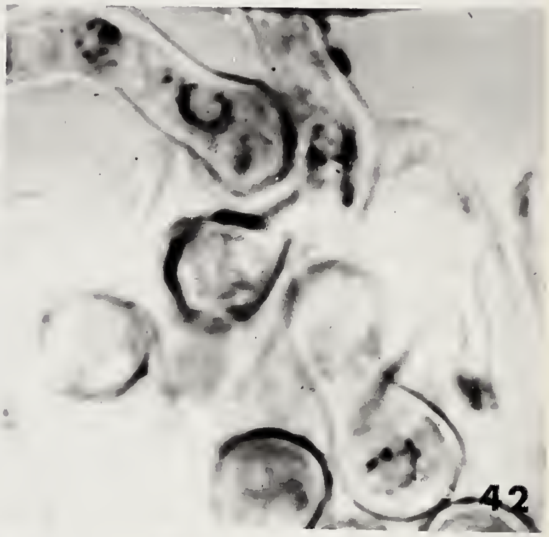

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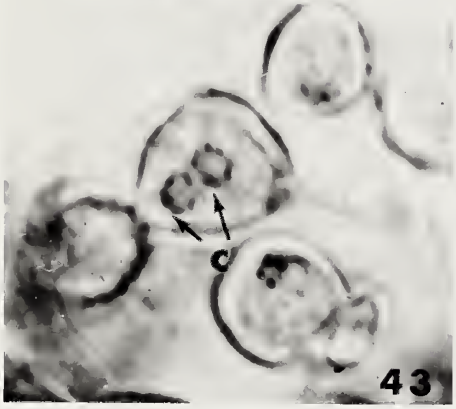

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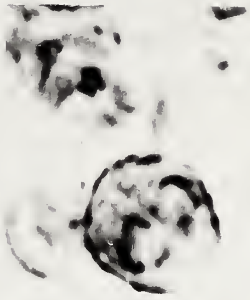


44

Plate VII, Figs. 45-51. Conidia of A. nidulans (8 hours of incubation).

Fig. 45 -- Undivided filament in resting conidium.

Fig. 46 -- Conidium with 'horseshoe-shaped' nucleus (center).

Fig. 47 -- Germinated conidium containing 'horseshoe-shaped' nucleus with attached centriole.

Fig. 48 -- Germinated conidium containing filamentous nucleus with divided centriole.

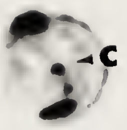
Fig. 49 -- Germinated conidium (left) with completely divided filamentous nucleus or an undivided 'horseshoe-like' nucleus.

Fig. 50 -- Young hypha with partially divided filamentous nucleus.

Fig. 51 -- Crescent shaped daughter nucleus in the hypha with the other daughter nucleus (in different focal plan) in the conidium.

Mag. x 4000

C = centriole



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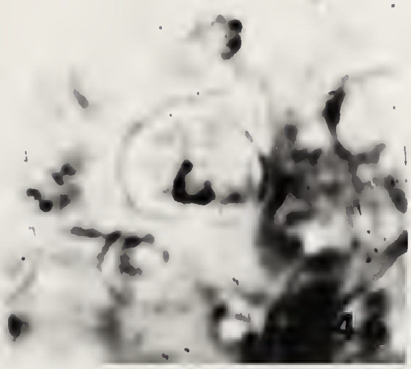

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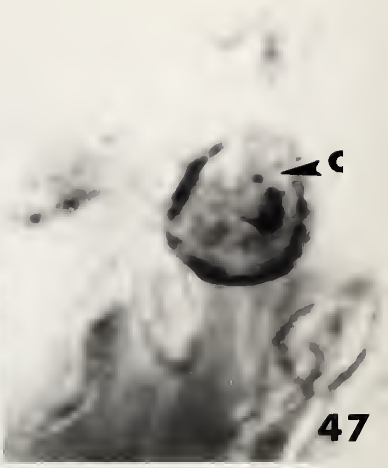

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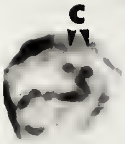

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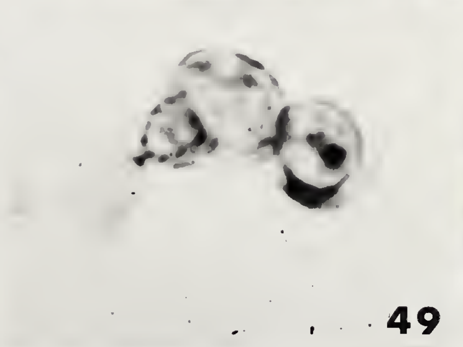

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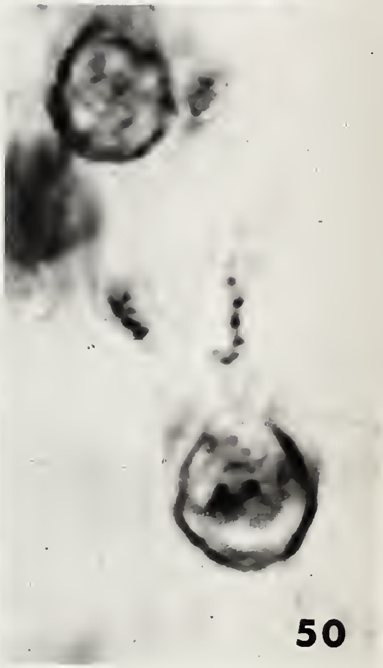

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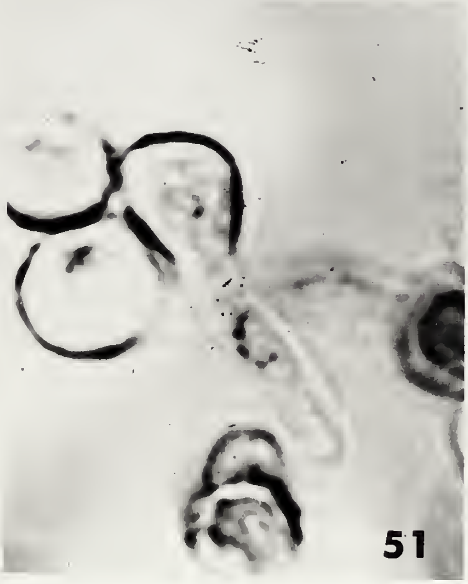


51

Plate VIII, Figs. 52-57. Conidia of A. nidulans (8 hours of incubation).

Fig. 52 -- Conidium with "figure-eight" shaped nucleus or two daughter ring nuclei.

Fig. 53 -- Germinating conidium (upper left) containing daughter crescent-like nuclei within the conidium.

Fig. 54 -- Germinating conidium (center) with daughter ring nuclei moving into the hypha.

Fig. 55 -- Germinating conidium containing daughter crescent-like nuclei.

Fig. 56 -- Germinating conidium containing daughter ring nuclei, one within the conidium and the other in the hypha.

Fig. 57 -- Ring nucleus and crescent-shaped nucleus within a single hypha.

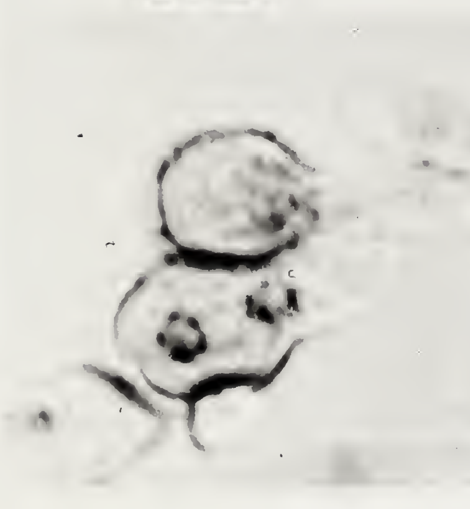
Mag. x 4300

52

53

54

55



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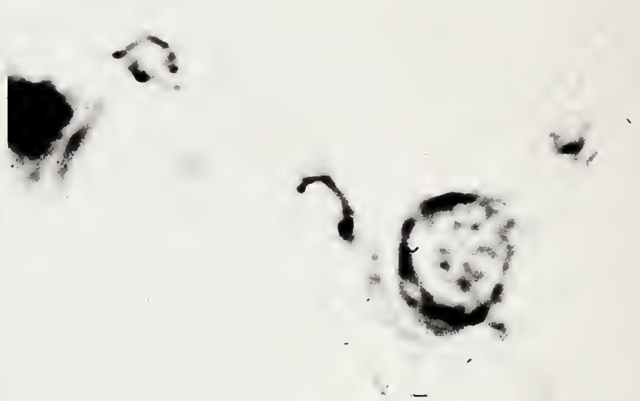

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Fig. 60 -- Schematic diagram of the Juvenile somatic chromosome cycle of *Aspergillus nidulans*.

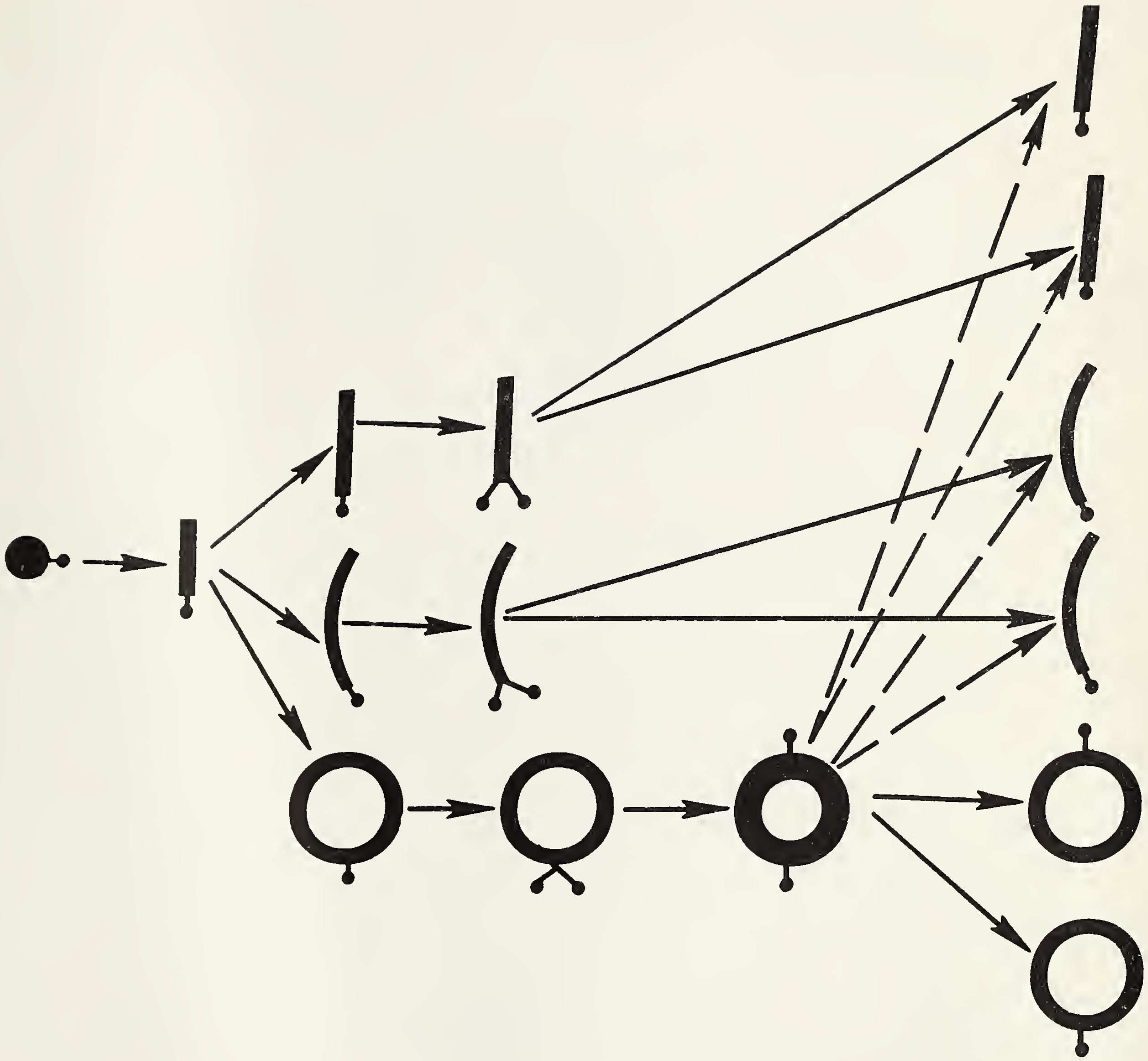


Fig. 60

Plate IX, Figs. 58, 59, 61 and 62. Conidia of A. nidulans (8 hours of incubation).

Fig. 58 -- Germinating conidium with ring nucleus (bottom right), and filamentous nucleus (center left).

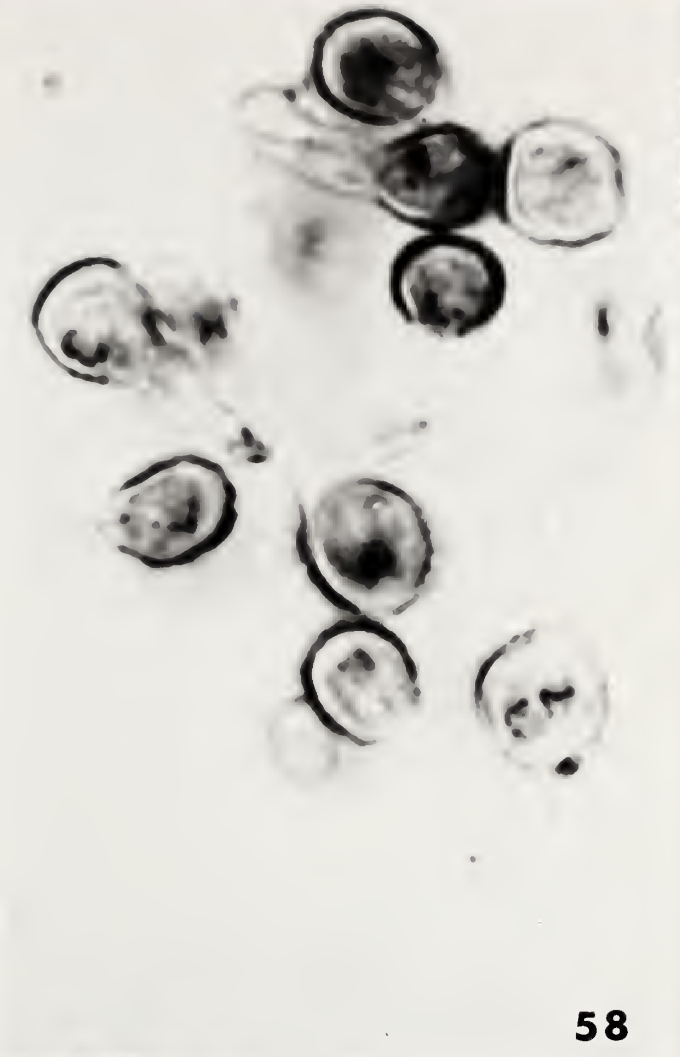
Fig. 59 -- Young hyphae with ring nuclei (center and bottom).

Fig. 61 -- Young hypha with 'horseshoe-like' nucleus (bottom), and dividing filamentous nucleus or possible 'broken' ring nucleus (center left).

Fig. 62 -- Germinating conidium with twisted, dividing filament.

Mag. x 4300

C = centriole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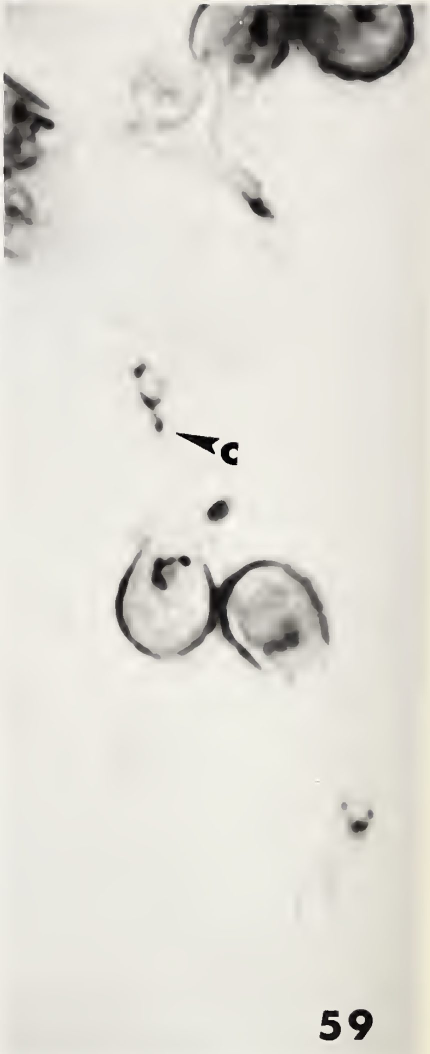

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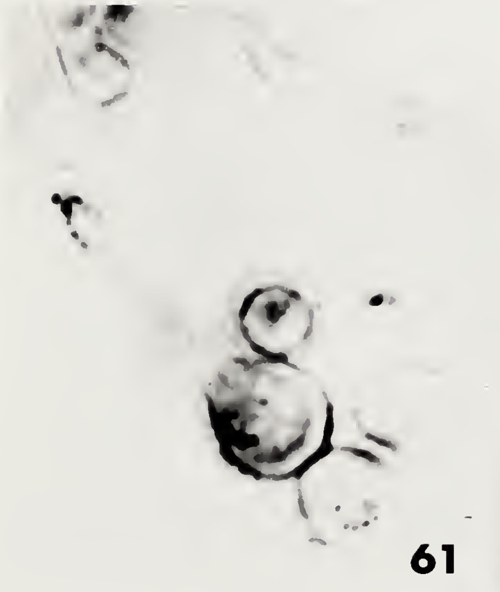
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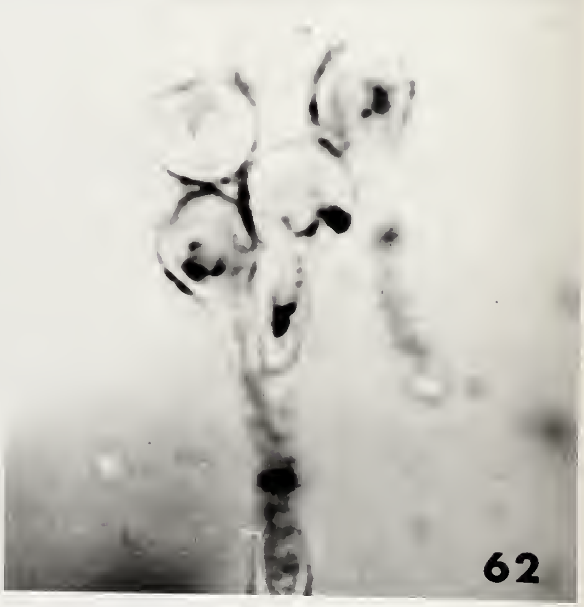
62

Plate X, Figs. 63-67. Conidia of A. nidulans (8 hours incubation).

Fig. 63 -- Germinating conidium with 'S-like' nuclear structure.

Fig. 64 -- Young hypha with partially divided filament (far right) and other hyphae containing variously shaped nuclei.

Fig. 65 -- Germinated conidium containing 'horseshoe-like' nucleus of 9 chromatin bodies.

Fig. 66 -- "Figure-eight" shaped nucleus in germinating conidium.

Fig. 67 -- Ring nucleus in germinated conidium (top) and other conidia with variously shaped nuclei. Note darkness of conidial walls in relation to the hyphal walls.

Mag. x 4300

C = centriole

63

64

65

66

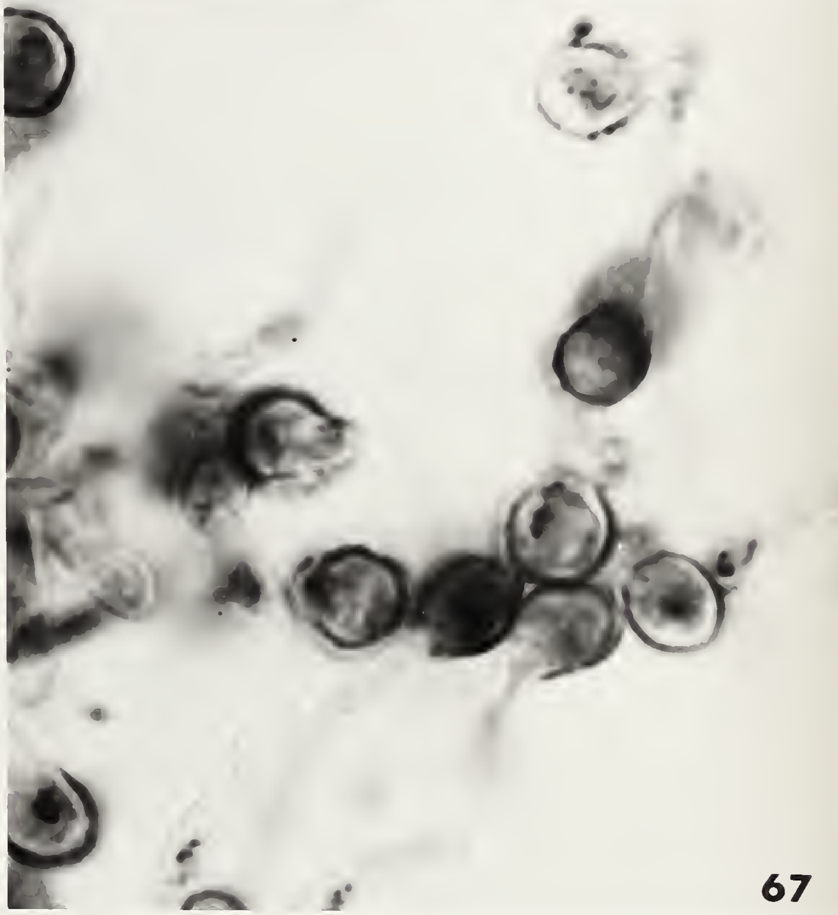

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Plate XI, Figs. 68-70. Conidia of _A. nidulans_ (10 hours of incubation).

Fig. 68 -- Young hyphae with non particulate ring nuclei (center) and nucleus with attached centriole (bottom center), filamentous nucleus (center left) and associated ring and filamentous nuclei (bottom left).

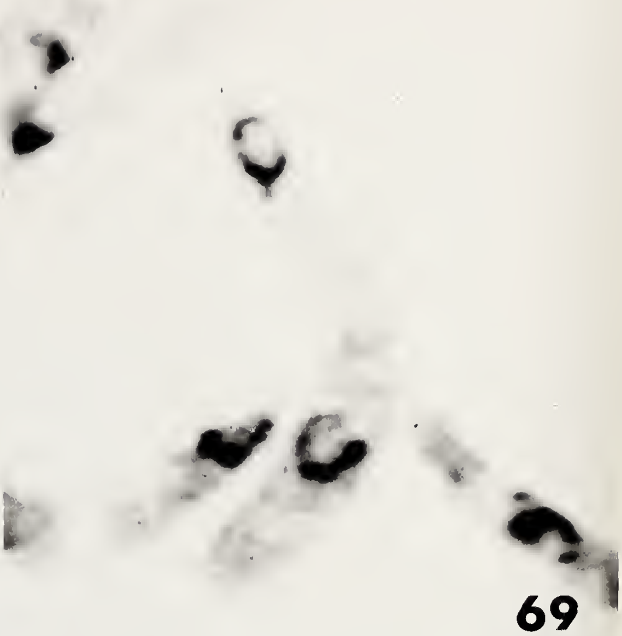
Fig. 69 -- Young hyphae with non particulate ring and partial ring nuclei.

Fig. 70 -- Young hyphae containing non particulate rings with associated incorporated centriole (center).

Mag. x 4800

C = centriole

68



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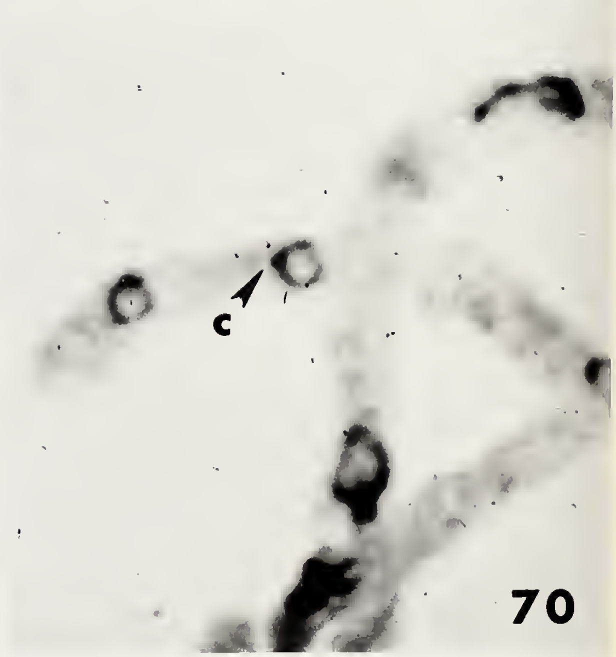


70

Plate XII, Figs. 71-75. Conidia of A. nidulans (12 hours incubation).

Fig. 71 -- Young hyphae with nuclear rings and filaments. Dividing nucleus giving rise to a ring and filament (center).

Fig. 72 -- Young hypha with dividing filament (center).

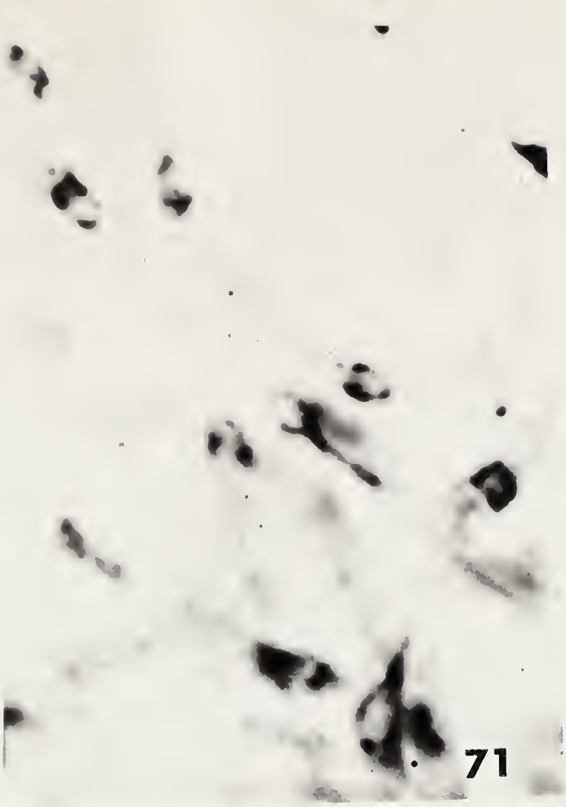
Fig. 73 -- Young hyphae with dividing filaments (top right and top left).

Fig. 74 -- Young hypha with non-particulate ring nucleus.

Fig. 75 -- Young hypha with nuclear ring structure associated with nuclear filament.

Mag. x 4300

C = centriole



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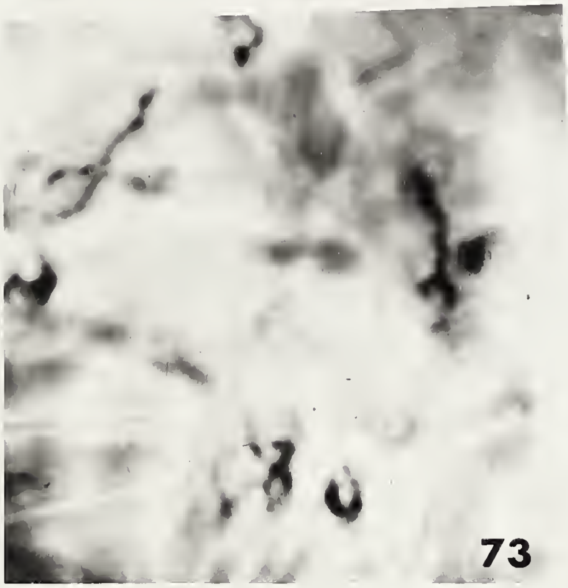

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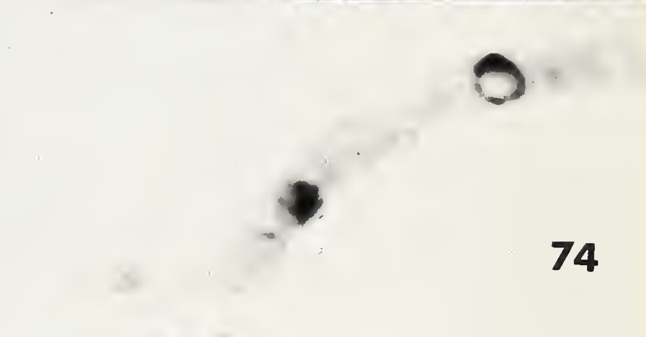

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75

Plate XIII, Figs. 76-82. Conidia of *A. nidulans* (14 hours of incubation).

Fig. 76 -- Mature hyphae with partially condensed nuclei.

Fig. 77 -- Hypha containing nuclei in various stages of condensation.

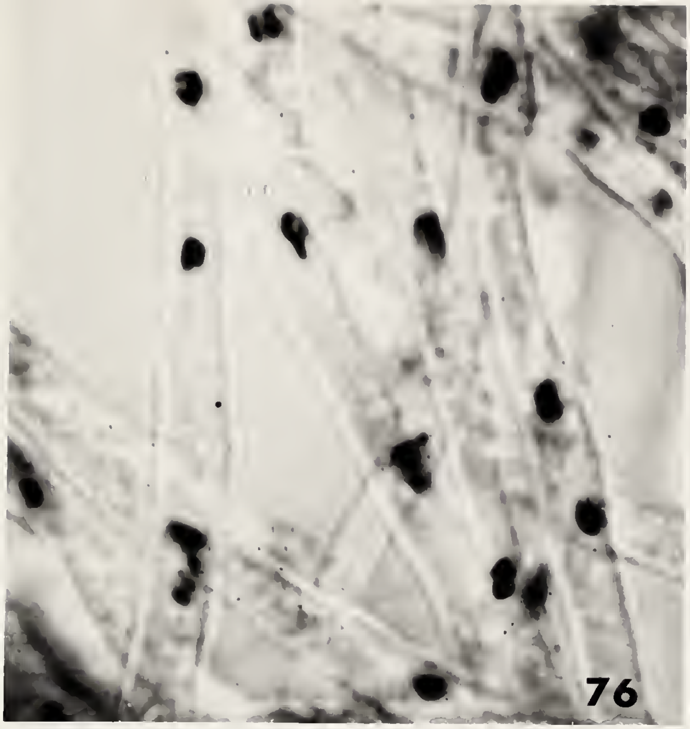
Fig. 78 -- Hypha containing partially condensed nuclei (nucleus appears to contain 4 bodies (center)).

Fig. 79 -- Hypha containing very condensed, spherical nuclei.

Fig. 80 -- Hypha containing condensed nuclei (note: nucleus with centriole, karyosome).

Fig. 81 -- Hyphae containing partially condensed nuclei.

Fig. 82 -- Hypha containing large filamentous nucleus with large triangular centriole (Maturation Cycle).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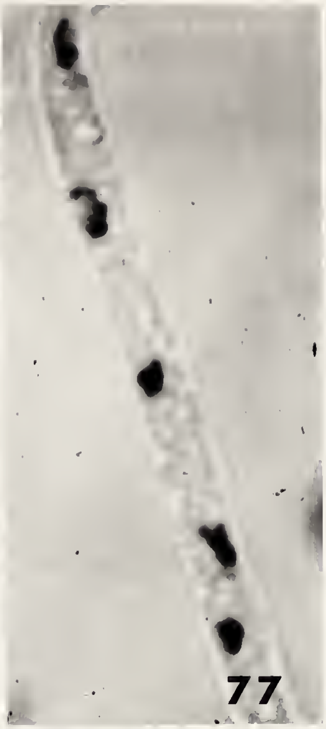
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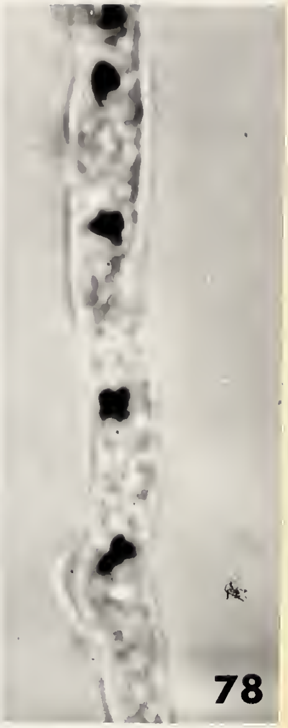
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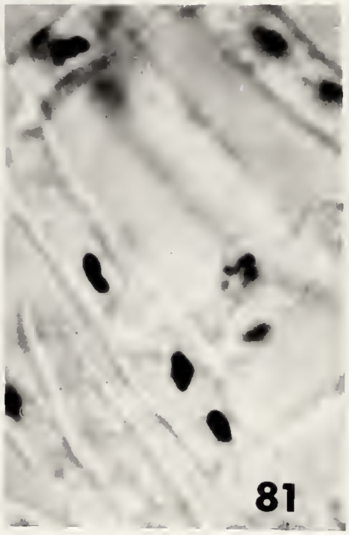
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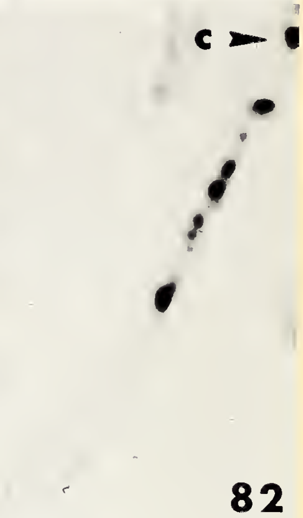

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